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(54) Procedure and apparatus for chromatographic separation.

(57) The invention concerns a procedure and an apparatus for chromatographic separation. According to the invention, the separation between a stationary, preferably liquid phase in a column and a gaseous mobile phase passed through it is performed in two stages by taking the sample under analysis to a first column (1) for rough separation of the compounds contained in it and cutting off the rough fraction separated from the sample by said column into a second column (2) for a second separation stage. The essential feature of the invention is that one or more reference compounds, i.e. retention index standards, are introduced into the first column (1) simultaneously with the sample, said reference compounds being so chosen that the retention time of at least one of them is shorter than that of the fraction to be separated from the sample, that the separation taking place in the first column is monitored using a detector (14), and that after the detector has detected one or more of the reference compounds said fraction is cut off into the second column (2) at an instant determined on the basis of the retention times of the detected reference compounds or other retention parameters calculated from the retention times. The procedure enables the cutting-off to be performed at the right moment regardless of the change in retention times caused by the soiling of the columns.

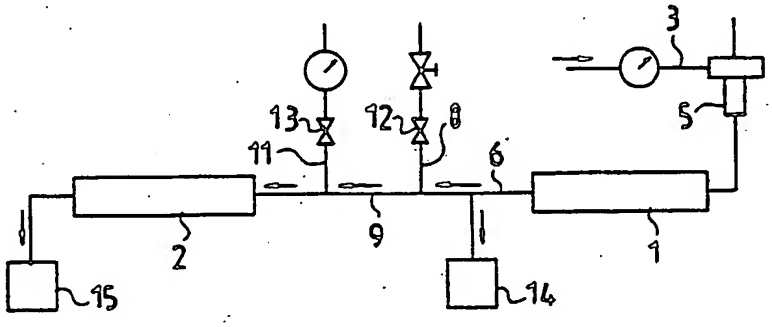

Fig. 2

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PROCEDURE AND APPARATUS FOR CHROMATOGRAPHIC SEPARATION

The present invention relates to a procedure for chromatographic separation whereby the separation between a stationary phase in a column and a mobile phase passed through it is performed in two stages by taking the sample under treatment into a first column for rough separation of the compounds contained in the sample and passing the rough fraction separated from it by said column into another column for a second separation stage.

Chromatographic separation is based on the partition or sorption occurring between a stationary phase and a mobile phase, which has the effect that the compounds contained in the sample are divided into fractions that move through the column at different speeds. The stationary phase may be either liquid or solid, whereas the mobile phase may be either gaseous or liquid. In typical gas chromatography, the mobile phase is a gas and the stationary phase a viscous liquid. The compounds or fractions separated in the column are generally identified on the basis of the retention times, using a detector.

Chromatographic separation can be implemented as a one-stage process, which, however, has the drawback of a poor separation capability. E.g. in industrial process supervision or in organic microelement analysis, the object under chromatographic separation may be a sample containing tens, hundreds or even thousands of different compounds in very low concentrations, e.g. of the order of a few ppm. Separating the compounds would require very long analysing times, which are not possible in repeated routine analyses.

Another common problem in chromatographic separation is that the column tends to get dirty, resulting in changes in the retention times of the compounds in the column. This again leads to difficulties in the identification of the separated compounds. In an attempt to solve this problem, reference compounds, called retention index standards, are passed through the column. On the basis of these, it is possible to estimate the changes which have occurred in the retention times of the compounds contained in the sample to be analyzed. However, this expedient does not provide sufficient help in cases where complex samples are to be analyzed, nor does it eliminate the problem of poor separation capability of the column.

Chromatographic analysis of complex samples can be rendered considerably more efficient by performing the separation in two stages in such manner that a first column separates a rough fraction of the sample, whereupon this fraction is cut off and passed to a second column for a definitive separation of the compound under analysis. In this case, the conditions prevailing in the two columns must be different, and this is achieved e.g. by using different substances as stationary phases in them. However, due to the changes in retention time resulting from the soiling of the column, it has been difficult in practice to determine the instant at which the desired fraction should be cut off.

The object of the present invention is to achieve a two-stage procedure for chromatographic separation based on the use of two successive columns that eliminates the above-mentioned drawback. The procedure of the invention is characterized in that one or more reference compounds are introduced into the first column simultaneously with the sample, said reference compounds being so chosen that the retention time of at least one of them is shorter than that of the fraction to be separated from the sample, that the separation taking place in the first column is monitored using a detector, and that, after the detector has detected one or more of the reference compounds, said fraction is transferred to the second column at an instant determined on the basis of the retention times of the detected reference compounds or on the basis of other retention parameters calculated from the retention times.

The invention eliminates the need for carrying out tests on the sample prior to analysis to determine the instant of cut-off. The procedure provides high selectivity, speed and repeatability and permits automatiztion of the routine treatment of similar samples.

The invention is especially applicable in gas chromatography, where the stationary phases in the columns are mutually differing liquids and the mobile phase in both columns is the same gaseous substance. The samples to be analyzed may be liquids, in which case they are vaporized, using an injector, into the mobile phase passed into the first column. Simultaneously, the reference compounds, which may have been mixed in advance with the sample to be injected, are mixed with the mobile phase.

According to the invention, the reference compounds, i.e. retention index standards, may constitute a homologous series of compounds which are similar to the compounds to be separated from the sample under analysis or to which the latter are otherwise suitably comparable. In principle, even one reference compound may be sufficient for the realization of the invention, but in practice the best results are achieved by using a series of compounds of which the first two members are obtained from the first separation stage before the

fraction to be cut off and one or more members after it.

The invention also relates to an apparatus for chromatographic separation, comprising two successive columns known in themselves, each of which contains filling material constituting the stationary phase, supply and discharge ducts connected to the first column for passing the mobile phase and the sample under analysis through the column for rough separation of the compounds contained in the sample, and an inter-column connecting duct for transferring the rough fraction separated from the sample by the first column into the second column for the second stage of analysis. The apparatus is characterized in that a detector is provided between the columns, allowing the separation taking place in the first column to be so monitored as to permit the transfer of said fraction to be based during analysis on the reference compounds detected by the detector, the reference compounds being introduced into the first column together with the sample.

The filling materials contained in the columns of the apparatus of the invention may be mutually differing liquids, e.g. polysiloxanes. The detector may be e.g. a flame ionization detector, an electron capture detector or a nitrogen-phosphorus detector.

In the following, the invention is described in detail by the aid of an example by referring to the attached drawing, in which

fig. 1 represents an apparatus for chromatographic separation as provided by the invention, consisting of two successive columns, during the first (or second) separation stage, and

fig. 2 represents the same apparatus during the cutting-off of the rough fraction separated from the sample during the first stage.

The separation equipment presented in figs. 1 and 2 comprises two successive columns 1, 2. In the first one 1 of these, the filling material is a viscous liquid, e.g. methyl polysiloxane, constituting the stationary phase, while the stationary phase in the second one 2 is another liquid, e.g. cyanopropyl polysiloxane. A gaseous mobile phase is supplied into the first column 1 via a feed duct 3. The feed duct is provided with a pressure gauge 4 and an injector 5 for the evaporation of the sample to be analyzed and the reference compounds or retention index standards into the gas flowing into the column 1. Connected to the discharge end of column 1 is a duct 6 which branches off at junction 7 into a discharge duct 8 of column 1 and an interconnecting duct 9 between columns 1 and 2. Via junction 10, the interconnecting duct 9 communicates with a feed duct 11 through which a gaseous mobile phase can be supplied into the second column 2 and, if necessary, also into the discharge duct 8 of column 1 via the interconnecting duct 9. The discharge duct 8 and said feed duct 11 connected to the interconnecting duct 9 are provided with valves 12, 13 enabling the ducts to be opened and closed. For the monitoring and control of the separation process, the apparatus is provided with detectors 14, 15, e.g. flame ionization detectors, one being placed between the columns 1, 2 in conjunction with the duct 6 leading away from column 1 and the other at the discharge end of column 2. The strength of the ionization current measured by the detector indicates the material quantity of the fraction eluted from the column. However, other types of detector can also be used, and the detectors 14, 15 may also be of different types if necessary.

The object under chromatographic separation treatment using an apparatus as illustrated by the drawing may be e.g. a sample of crude oil in which certain hydrocarbons are to be determined. The mobile phase consists of a carrier gas, which may be e.g. hydrogen, nitrogen or helium. The sample and the reference compounds, which may consist of a homologous series of hydrocarbons of a certain type, are injected at a given instant into the carrier gas, which is supplied as a continuous flow via duct 3 into column 1 and further into the discharge duct 8, the valve 12 in this duct being open. Simultaneously, a continuous flow of carrier gas is supplied via feed duct 11 into the second column 2 and through the interconnecting duct 9 into the discharge duct 8 of column 1. These flows are indicated by arrows in fig. 1.

The carrier gas flow in feed duct 3 conveys the sample and the reference compounds into the first column 1, where, as a result of the partition occurring between the stationary phase and the carrier gas, the sample is divided into fractions that advance in the column at different speeds. Each fraction remains in the column for a given retention time until it reaches the discharge end. The flux flowing from column 1 into discharge duct 8 is monitored by detector 14, which is specially aligned so that it will detect the first reference compounds emerging from the column.

On the basis of the retention indexes calculated from the retention times of the reference compounds detected by detector 14, it is possible to determine with considerable accuracy the instant at which the desired fraction separated from the sample by the first column 1 must be cut off and passed into the second column 2 for further separation. Detector 14 can be so connected to the valves 12, 13 that these will be automatically closed at the instant of cut-off. The flow from the first column 1 will then proceed, as illustrated by the figure, into the second column 2, where the rough fraction will be finally partitioned into separate compounds.

Once the cut-off has occurred, the valves 12, 13 in the discharge duct 8 and feed duct 11 are opened, thus permitting the carrier gas to flow again from the feed duct 11 into the second column 2 and via the interconnecting duct 9 into the discharge duct 8. Thus, the situation is again as illustrated by fig. 1. As the carrier gas is flowing into the second column 2, the fraction which was cut off is partitioned into individual compounds which, after certain retention times, are detected by detector 15.

It is obvious to a person skilled in the art that different embodiments of the invention are not restricted to the example described above, but that they may instead be varied within the scope of the following claims.

Reference signs in the claims are intended for better understanding and shall not limit the scope.

Claims

1. Procedure for chromatographic separation, whereby the separation between a stationary phase in a column and a mobile phase passed through it is performed in two stages by taking the sample under treatment to a first column (1) for rough separation of the compounds contained in the sample and passing the rough fraction separated from the sample by said column into a second column (2) for a second separation stage, **characterized** in that one or more reference compounds are introduced into the first column (1) simultaneously with the sample, said reference compounds being so chosen that the retention time of at least one of them is shorter than that of the fraction to be separated from the sample, that the separation taking place in the first column is monitored using a detector (14), and that after the detector has detected one or more of the reference compounds said fraction is transferred to the second column (2) at an instant determined on the basis of the retention times of the detected reference compounds or on the basis of other retention parameters calculated from the retention times.

2. Procedure according to claim 1, **characterized** in that the stationary phases in the first and second columns (1, 2) are different substances, whereas the mobile phase in both columns consists of the same substance.

3. Procedure according to claim 2, **characterized** in that the stationary phases are liquids and that the mobile phase is a gas.

4. Procedure according to any one of the preceding claims, **characterized** in that the reference compounds comprise a homologous series of compounds.

5. Procedure according to claim 4, **characterized** in that the fraction separated by the first column (1) is transferred into the second column (2) after at least two of the reference compounds of the homologous series have been detected by the detector (14).

6. Apparatus for chromatographic separation, comprisisng two successive columns (1, 2) each of which contains filling material constituting the stationary phase, supply and discharge ducts (3, 6, 8) connected to the first column (1) for passing the mobile phase and the sample under analysis through the column for rough separation of the compounds contained in the sample, and an inter-column connecting duct (6, 9) for transferring the rough fraction separated from the sample by the first column into the second column (2) for a second separation stage, **characterized** in that a detector (14) is connected between the columns (1,2), allowing the separation taking place in the first column (1) to be so monitored as to permit the transfer of said fraction into the second column (2) to be based during analysis on the reference compounds detected by the detector, said reference compounds being introduced into the first column together with the sample.

7. Apparatus according to claim 6, **characterized** in that the filling materials contained by the columns (1, 2) are mutually differing liquids, such as polysiloxanes.

8. Apparatus according to claim 6 or 7, **characterized** in that the detector (14) is a flame ionization detector, an electron capture detector or a nitrogen-phosphorus detector.

9. Apparatus according to any one of claims 6-8, **characterized** in that the discharge duct (8) of the first column (1) and the connecting duct (9) between the columns (1, 2) branch off from each other and are provided with valve means (12, 13) enabling the flow discharged from the first column to be directed into either one of said ducts on the basis of a detection signal provided by the detector (14).

10. Apparatus according to claim 9, **characterized** in that the connecting duct (9) between the columns (1, 2) communicates with a feed duct (11) through which a substance constituting the mobile phase can be supplied into the second column (2) and also, via the connecting duct, into the discharge duct (8) of column (1).

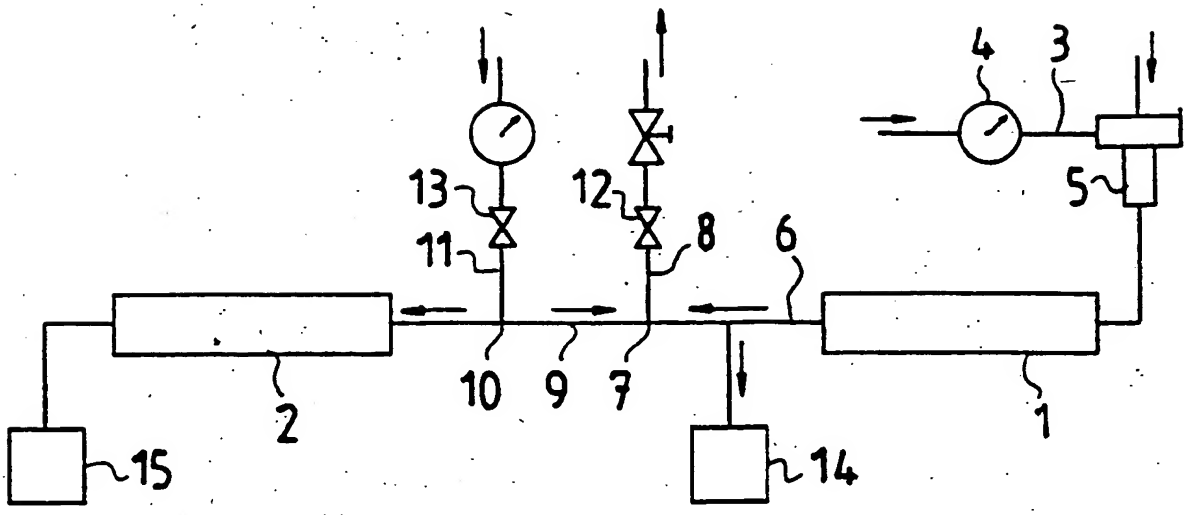

Fig.1

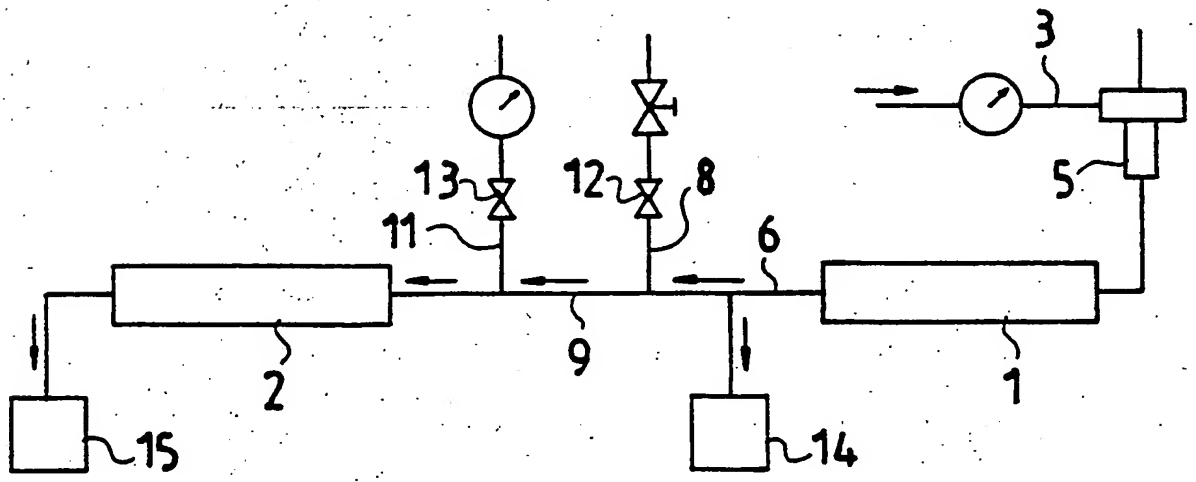

Fig. 2